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Volume 7
Number 100 *Holcus bacterial spot of Zea mays and Holcus species*
Article 1

November 1926

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Recommended Citation
Kendrick, James B. (1926) "Holcus bacterial spot of Zea mays and Holcus species," *Research Bulletin (Iowa Agriculture and Home Economics Experiment Station)*: Vol. 7 : No. 100 , Article 1.
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November, 1926 Research Bulletin No. 100

HOLCUS BACTERIAL SPOT OF ZEA MAYS AND HOLCUS SPECIES

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HOLCUS BACTERIAL SPOT OF ZEA MAYS AND HOLCUS SPECIES

By James B. Kendrick[1]

A bacterial leaf spot disease on corn (*Zea mays* L.) has been under observation in Iowa since 1916. In the fall of 1924, it was quite prevalent on *Zea mays*, and on volunteer sorghum (*Holcus sorghum* L.) growing together near Ames, Iowa. Further examination revealed the presence of a somewhat similar leaf spot on *Holcus sorghum*, sudan grass [*H. sorghum* var. *sudanensis* (Piper) Hitchc.], Johnson grass (*H. halepensis* L.) and pearl millet [*Pennisetum glaucum* (L.) R.Br.]. These observations led to a cultural study of the causal agents and a survey of the literature on bacterial diseases of these hosts.

As is well known, Burrill (1, 2) as early as 1887 described a bacterial disease of sorghum and broomcorn. This disease, however, attacked the roots and stems as well as leaves and leaf-sheath. Burrill characterized it as producing irregular red spots or blotches on the infected parts. The symptoms were especially noticeable on the upper leaf-sheath and along the veins and midribs of the leaves. The diseased roots were at first red, but later lost their bright color and soon decayed. Infected plants presented a stunted appearance and often died. In 1889 (3,4) the same author described a somewhat similar disease on corn, but attributed it to a different organism.

In 1888 Kellerman and Swingle (15) reported Burrill's disease of sorghum in Kansas. In 1905 Smith and Hedges (28) described a disease of broomcorn and sorghum as "Burrill's bacterial disease of broomcorn," but did not accept or reject the organism previously described by Burrill. In a later publication, Smith (29) briefly characterized the organism and named it *Bacterium andropogoni*, apparently rejecting the name *Bacillus sorghi* previously suggested by Burrill. In 1924 Elliott and Smith (10) further described the disease of broomcorn and sorghum caused by *Bacterium andropogoni* as producing rather elongated necrotic streaks on the leaves with the red discoloration thruout the infected area, accompanied by an abundant red exudate. They said that no natural infection was found on sudan grass, but typical long dark red streaks were produced in inoculation experiments which differed from the common leaf spot of sudan grass.

[1]This paper was presented to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree, Doctor of Philosophy. The writer wishes to acknowledge his indebtedness to Dr. I. E. Melhus, under whose direction this work was done, for helpful criticism and suggestions received thruout these investigations.

Bruyning (5) in 1898 described two species of chromogenic bacteria isolated from the red discoloration on the stalks and in the pith of sorghum as the cause of the condition known as "sorghum blight." More recently Rosen (23,24,25) has described a bacterial root and stalk rot on field corn, and a spot disease on foxtail (*Chaetochloa lutescens*) (26, 27) which he transferred to wheat, oats, rye, barley, corn and sorghum.

The symptoms of the spot disease under consideration differ quite markedly from any of the diseases described above, and the causal agent is unlike any reported on corn and sorghum. These facts led the author to undertake a study of the causal agent as to its pathogenicity, its host range, its identity and pathological changes induced in the host tissues.

HOST RANGE OF THE HOLCUS BACTERIAL SPOT

The hosts for this disease as determined by field observations are as follows: sorghum (*Holcus sorghum* L.), sudan grass [*Holcus sorghum* var. *sudanensis* (Piper) Hitchc.], broomcorn (*H. sorghum* var. *technicus* Bailey), Johnson grass (*H. halepensis* L.), pearl millet [*Pennisetum glaucum* (L) R. Br.], dent corn (*Zea mays* var. *indentata* Bailey), sweet corn (*Z. mays* var. *saccharata* Bailey) and foxtail [*Chaetochloa lutescens* (Weigel) Stuntz.].

Greenhouse inoculations have shown that flint corn (*Zea mays* var. *indurata* Bailey) and popcorn (*Zea mays* var. *everta* Bailey) are also hosts for this disease. A large number of seedsmen's varieties of *Holcus sorghum* and *Zea mays* have been infected artificially in the greenhouse.

A total of 22 seedsmen's varieties of *Holcus sorghum* have been found susceptible either in the field or by greenhouse inoculations as follows: Orange Cane, White Kafir, Red Amber, Black Amber, Milo, Standard Blackhull Kafir, Shallu, Shrock, Sunrise, Kaferita, Dwarf Hegari, Dwarf Kafir, Dwarf Sumac, Red Kafir, Sumac, Kansas Orange Black Glume, Pink Kafir, Jap Sugar Cane, Higeria, Feterita, and Ribbon Cane. All varieties tested have proved susceptible.

Holcus sorghum var. *sudanensis, H. sorghum* var. *technicus* and *H. halepensis* have proved to be hosts for the disease in the field and greenhouse. The disease has been observed in the field on *Pennisetum glaucum* and the same host has repeatedly responded to artificial inoculations in the greenhouse. A few spots were observed on *Chaetochloa lutescens* in the field and slight infection has been secured in the greenhouse, but indications are that it is highly resistant.

The disease was observed in the field on dent corn and sweet corn. The following varieties of dent corn were artificially in-

fected in the greenhouse: Reid's Yellow Dent, Wimple's Yellow Dent, St. Charles White, Champion White Pearl, Minnesota Number 13, Pickett's Yellow Dent, Boone County White, Lancaster Sure Crop, Golden Orange, Silver King, and Calico. Two varieties of flint corn, Rainbow Flint and Rhode Island White Flint, also responded to artificial greenhouse inoculations. The disease was observed in the field on the following varieties of sweet corn: Country Gentleman, Howling Mob and Early Crosby. In addition the varieties, Black Mexican and Narrow Grained, were artificially infected in the greenhouse. Popcorn was artificially infected under greenhouse conditions.

The following grass species failed to become infected under repeated greenhouse tests and the disease was not observed on them in the field where field observations were possible: Brome grass (*Bromus inermis* Leyss), English rye grass (*Lolium perenne* L.), phalaris grass (*Phalaris sp.*), meadow fescue (*Festuca elatior* L.), orchard grass (*Dactylis glomerata* L.), tall meadow oat grass (*Arrhenatherum elatius* L. Mert. and Koch.), timothy (*Phleum pratense* L.), red top (*Agrostis alba* L.), wheat (*Triticum vulgare*) and oats (*Avena sativa* L.).

The following millet [*Chaetochloa italica* (L.) Scribn.] varieties have failed to become infected artificially in the greenhouse: Japanese, Siberian, Common, Hungarian and Broom-corn.

SYMPTOMS OF THE DISEASE ON THE DIFFERENT HOSTS

ZEA MAYS

The disease has been observed only on leaves of this host. The spots occur on the lower leaves and are usually more numerous toward the tip. Incipient lesions are round, elliptical or irregular, dark green and water-soaked, and vary in diameter from 2 to 10 millimeters (fig. 2). Later the spots lose their water-soaked appearance and become brown-centered with a somewhat darker brown to reddish brown narrow border. When viewed by transmitted light a narrow yellowish halo is usually visible, especially around the larger lesions (fig. 2A). The darker spots in fig. 2B are incipient lesions among the numerous older lesions on the leaf.

When conditions are especially favorable for the spread of the organism, marginal infection develops along the entire edge of the lower leaves and causes a dark brown necrotic condition of the entire margin. This condition is brought about only during periods of rainy weather.

Infection was first noted in the field on the lower leaves, June 25, 1925, following a period of rainy weather. Incipient lesions were noted following rains, but none during periods of dry

weather. By July 20, lesions were found on leaves up to the seventh and eighth node.

The bacterial lesions should not be confused with round to irregular necrotic areas, common on leaves of *Zea mays* in practically all fields, which have a white papery appearance and no definite border. Repeated isolations and examinations failed to reveal any organism associated with such lesions.

The lesions resulting from artificial inoculations on *Zea mays* in the greenhouse were somewhat different from those observed in the field in that after they lose their water-soaked appearance they are smaller, light colored, papery necrotic spots with a narrow light brown border.

HOLCUS SORGHUM, H. SORGHUM VAR. SUDANENSIS AND *H. HALEPENSIS*

The symptoms of the disease on these three hosts are very similar. Under field conditions the spots first appear on the lower leaves and infection gradually spreads to the upper leaves as the plants approach maturity.

The lesions occur at any place on the leaf and vary in size from one to eight millimeters in diameter. The spots are usually round or elliptical, sometimes linear to irregular, of variable size, and at first dark green water-soaked (fig. 1, B and C). In a few hours after infection is visible, the red color appears, even before the spots lose their water-soaked appearance. The lesions soon become dry and assume a parchment-like appearance with a red border (brown in the case of Shallu, fig. 1, A). Often the smaller lesions are entirely red with a very small, somewhat sunken center. Different varieties of *Holcus* have varying degrees of red color associated with the lesions, while the variety known as Shallu has a dark brown border instead of a red.

Often marginal infection occurs, causing rather long necrotic areas along the edge of the leaf (fig. 1, A and C). The lesions are usually limited by the veins, but often the coloration extends across the veins and where several spots are close together they converge to form rather large necrotic areas on the leaf (fig. 1, A).

PENNISETUM GLAUCUM, CHAETOCHLOA LUTESCENS AND *C. ITALICA*

The disease on *Pennisetum glaucum* does not differ materially from that on the other hosts except in the color of the spots. The latter are usually a dark brown color with a light greenish halo. Marginal infection is more common on this than any of the other hosts, causing elongated necrotic areas on the edge of the leaf. The lesions on *Chaetochloa lutescens* and *C. italica* are very similar to those on *Pennisetum glaucum*, except that they are smaller. These hosts have shown only slight susceptibility.

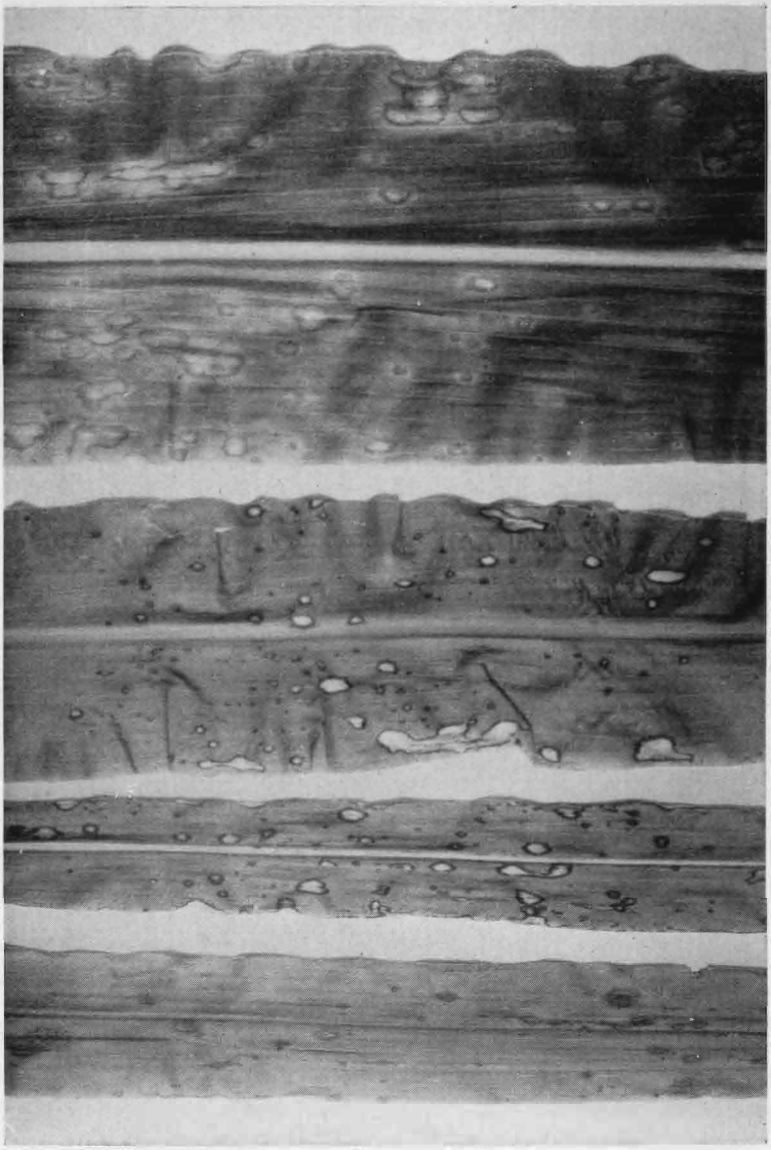

Fig. 1. Bacterial lesions on Holcus species and *Pennisetum glaucum* resulting from natural infection in the field. (Reading from top to bottom) A. Leaf of *Holcus sorghum* (variety Shallu) showing light centered, dark brown bordered lesions and rather large neurotic areas due to converging of spots. Also shows marginal lesions. B. Leaf of *H. Sorghum* (variety orange cane) showing various types of lesions. Some are small red sunken dots, others are larger, round, oblong to irregular with parchment-like center and light red border. C. Leaf of *H. sorghum* var. *sudanensis* showing lesions similar to B. D. Leaf of *Pennisetum glaucum* showing dark brown round to oblong lesions of various sizes.

NAME OF THE DISEASE

The name "Sorghum blight" has been commonly applied to the red spot disease of sorghum since the early work of Burrill (1, 2) in which he designated the causal agent as *Bacillus sorghi*. Smith and Hedges (29, 28) designated what was apparently Burrill's "Sorghum blight" as "Burrill's bacterial disease of broomcorn," but considered the causal agent to be a different bacterium which they named *Bacterium andropogoni*. It is not now possible accurately to identify the organism which Burrill described as the cause of "Sorghum blight," and it is evident that the pathological condition which he described was partially due to a combination of unfavorable environmental conditions and saprophytic organisms.

The organism causing the disease under consideration in this publication differs materially from *Bacillus sorghi* and *Bacterium andropogoni*. The symptoms of the disease are also quite different from the "Sorghum blight" of Burrill as well as the disease designated by Smith and Hedges (29, 28) as "Burrill's bacterial disease of broomcorn." Considering these differences, which will be enumerated more fully elsewhere in this publication, together with the fact that Burrill's "Sorghum blight" is a term which was earlier applied to what was apparently a combination of pathological conditions, the name "Holcus bacterial spot" is suggested for the disease described herein.

STUDIES OF THE CAUSAL ORGANISM

Numerous isolations were made from natural infection of the different hosts having the symptoms described above and reisolations were made from artificial infection in the greenhouse. Several strains of the organism secured from the different hosts were compared in an extensive morphological and physiological study. These studies showed the organisms from these different hosts to be the same.

ISOLATIONS FROM *ZEA MAYS*

September 24, 1924, a large number of isolations were made from conspicuous light centered, dark bordered spots on leaves and leaf-sheaths of *Zea mays* collected in the experimental plots of the Farm Crops Department, at Ames, Iowa. These isolations were made by surface-sterilizing the tissues in mercuric chloride 1 to 1000 and then washing in sterile water. The tissue fragments were crushed in a drop of sterile water on a flamed slide and dilution plates made in potato dextrose agar. In 48 hours, a number of the plates from the leaf lesions showed an abundance of bacterial colonies, some yellow and some white. Transfers were made of single isolated colonies of both the white and the yellow organisms. Subsequent inoculations on *Zea mays, Holcus*

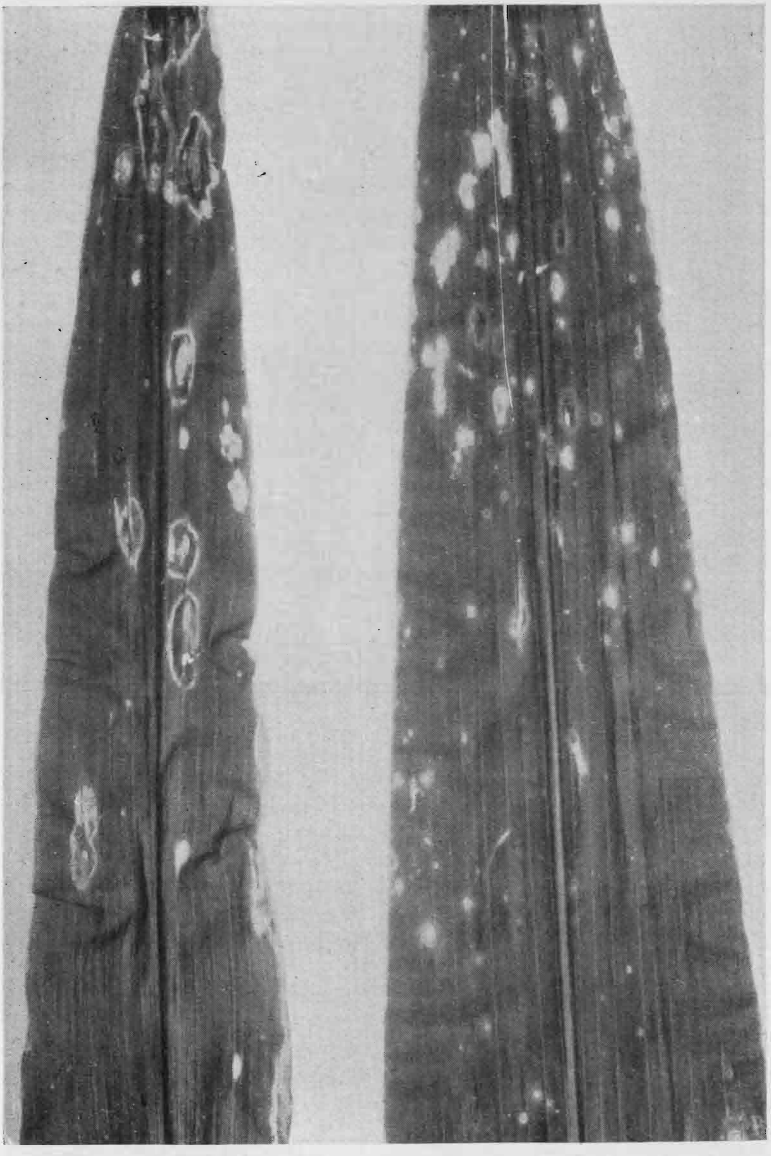

Fig. 2. Tips of lower *Zea mays* leaves showing the lesions resulting from natural infection in the field. A. (left) Leaf showing small and large lesions as well as necrotic marginal areas. The brown centers with darker brown border and narrow halo are evident in larger lesions. The smaller light colored lesions resemble somewhat the lesions produced artificially in the greenhouse. B. (right) Old and incipient lesions of various size on the same leaf. The darker areas are incipient lesions and are dark green water-soaked with a narrow light colored halo.

sorghum and *H. sorghum* var. *sudanensis*, in the greenhouse with the two organisms showed that only the white organism was pathogenic. Many different organisms, not including the one under consideration, were secured from the corn leaf-sheath spots which is in accord with Durrell's (6) findings.

October 20, 1924, dilution plates were made from *Zea mays* leaf spots similar to those mentioned above. In these plates white bacteria of a uniform type developed, which resembled the organism secured from the previous isolations and which also proved pathogenic to *Zea mays* and *Holcus sorghum* in the greenhouse.

On June 25, 1925, following a period of rainy weather, spots were found on the lower leaves of sweet corn (var. Country Gentleman) in a field near Ames, which were apparently bacterial in nature and resembled the spots noted in the fields of corn in the fall of 1924. Similar leaf lesions were noted in four other fields near Ames. A large number of isolations were made from these lesions and a white fluorescent bacterial organism was consistently secured which resembled the organism previously isolated from *Zea mays* and *Holcus sorghum*, and which proved pathogenic to *Zea mays* and *Holcus sorghum* by subsequent inoculation tests in the greenhouse.

ISOLATIONS FROM *HOLCUS* SPP., *PENNISETUM GLAUCUM* AND *CHAETOCHLOA LUTESCENS*

In a corn field near Ames in September, 1924, a round to elliptical red or light centered and red bordered spot was noted on leaves of volunteer *Holcus sorghum* plants. Leaves showing these spots were brought in and examined under the microscope by cutting the lesions in a drop of water on a slide. Bacteria oozed from the tissues from many of the necrotic areas. Isolations were made using the method previously described and in two days white bacterial colonies of a uniform type appeared in the plates which resembled the previous isolations from spots on *Zea mays*. Transfers were made to agar slants and the pathogenicity of the organism proved by inoculation.

An examination of the Farm Crops Department experimental plots of October 24, 1924, showed the presence of a similar leaf spot on *Holcus sorghum* and *H. sorghum var. sudanensis*. A round elliptical to irregular spot of apparently bacterial nature was also noted on *Pennisetum glaucum* growing in the same plot. A white bacterial organism similar to the one discussed above was isolated from the above named plants and its pathogenicity determined. A killing frost occurred thruout Iowa about this time and prevented further work on field material until the spring of 1925.

A few *Holcus sorghum* heads were collected in the field of *Zea*

mays previously mentioned near Ames. Seeds from these heads were planted in pots in the greenhouse to furnish plants for inoculation. When the plants were very small, light centered, red bordered, round to elliptical spots were noted on the margin or near the center of several of the first leaves. These spots resembled some of those noted on the *Holcus sorghum* leaves in the field. The spots were surface-sterilized with mercuric chloride, rinsed in sterile water and cut in a drop of sterile water on a flamed slide. Bacteria oozed from the tissues. Isolations were made and a white organism secured which appeared to be the same as the organism previously isolated from *Zea mays* and *Holcus sorghum*.

On June 26, 1925, a brown, round to irregular spot was noted on a few leaves of *Chaetochloa lutescens* growing near plants of sweet corn. Examination under the microscope revealed bacteria in the tissues and isolations and subsequent inoculations showed the organism to be the same as previously isolated from *Zea mays* and *Holcus sorghum*.

July 27, 1925, 30 isolations were made from *Zea mays, Holcus sorghum, H. sorghum var. sudanensis, H. halepensis* and *Pennisetum glaucum* leaf lesions collected in the field. The lesions were surface-sterilized and the isolations made in the usual way. All plates showed an even and abundant seeding of apparently the same white fluorescent bacterial organism as that secured in previous isolations. Transfers were made and the pathogenicity of the cultures proven.

MORPHOLOGY OF THE CAUSAL ORGANISM

The organism causing holcus bacterial spot is a short rod with rounded ends, usually solitary, but sometimes occurring joined together in pairs. The bacteria stain readily with Ziehl's carbol fuchsin or anilin gentian violet. The size of the organism from 24-hour to 4-day-old cultures shows little variation. When stained with Ziehl's carbol fuchsin and anilin gentian violet, the cells measured from 0.6 to 1 μ in width and 1.5 to 2.9 μ in length with an average of 0.72 by 2.12 μ.

The organism is motile by means of polar flagella (1-4) at one pole only, the usual number being one or two. (Fig. 4.) For flagella staining, a two millimeter loop of growth from a 20 to 24-hour agar slant was removed and placed in a 10 c.c. water blank which was permitted to stand several hours to allow the motile bacteria to diffuse thruout the water. A two-millimeter loop was then removed and carefully smeared on a clean cover glass and stained by Plimmer's method.

Endospores or involution forms have not been observed and the presence of capsules have not been demonstrated. The organism is gram-negative.

CULTURAL CHARACTERS

The organism grows readily on the ordinary laboratory culture media, but markedly better when one percent dextrose is added. For general laboratory use neutral (pH7) potato agar and beef-peptone agar containing one percent dextrose were found to be most satisfactory. The reaction of all culture media was adjusted to pH 7.0 with normal sodium hydroxide or normal hydrochloric acid.

Inoculations were made from heavy water suspensions made by removing the growth from a 24 to 36-hour-old agar slant to a 10 c.c. water blank. Unless otherwise noted, all cultures were incubated at room temperature. Strains of the organism isolated from *Holcus sorghum*, *H. sorghum* var. *sudanensis*, *Zea mays* and *Pennisetum glaucum* were carried in parallel series. These cultures were purified by poured plates and their pathogenicity tested before being used. A culture of *Bacterium coli* was carried in parallel series in most of the tests.

Agar poured plates. On poured plates of beef-peptone agar, small, circular, white fluorescent colonies appeared in 24 hours. In 36 to 40 hours, the surface colonies were round, convex, one to two millimeters in diameter. Surface colonies reached their maximum growth in four to five days, and were two to four millimeters in diameter; margin entire; surface smooth; amorphous, viscid, grayish-white in reflected light and slightly greenish-yellow fluorescent in transmitted light. Submerged colonies were lens-shaped, white and very small. The agar was unchanged in color.

On poured plates of potato agar containing one percent dextrose, growth was similar to that on beef-peptone agar except that it was more vigorous. Colonies appeared in 24 hours and by 36 to 40 hours were one to three millimeters in diameter. The medium was unchanged in color and no odor was present.

Agar stabs. Stab cultures on beef-peptone agar showed best growth at the top. There was slight growth along the line of puncture near the surface only. In four days, the growth had spread over the entire surface with a raised area around the point of puncture. A slight greenish-yellow pigment was produced which diffused thru the medium to a depth of three centimeters from the surface.

Agar slants. Slant cultures on beef-peptone agar showed moderate growth in 24 hours, filiform, slightly raised along the edges, smooth surface, grayish white color in reflected light and greenish fluorescent in transmitted light, especially along the edges. The cultures reached their maximum growth in three days and never entirely covered the surface of the slant. There was slight evidence of a greenish-yellow pigment which diffused thru the medium directly under the slant. The medium was otherwise unchanged.

Cultures on slants of potato dextrose agar showed more abundant growth than on beef-peptone agar, but otherwise appeared the same.

On slants of beef-peptone agar containing one percent dextrose, growth was more abundant than on the other slanted media. In three days the entire surface of the slant was covered with a slightly raised growth of much the same appearance as on the other media.

Cultures on veal infusion agar slants made only moderate growth. Slightly more evidence of the greenish-yellow pigment was produced on this medium than on the other agar slants.

Gelatin plates. Small, circular colonies appeared on poured plates of plain nutrient gelatin incubated at 20° C. in 24 to 36 hours. Liquefaction was evident in 36 hours by the round colonies being present in small saucer-shaped cavities. In 48 hours the entire medium was liquefied.

Gelatin stabs. Stab cultures on plain nutrient gelatin were held at 20° C. Growth in 24 hours was best on the surface and extended down the line of puncture to the bottom of the tube. Liquefaction occurred in a shallow crateriform area on the surface. Liquefaction progressed rather rapidly, later becoming striated, and at the end of seven days liquefaction was infundibuliform to striated with a white flocculent precipitate at the bottom of the liquid portion of the medium. The medium was practically half liquefied at the end of 10 days-.

Potato cylinders. Growth on steamed potato cylinders was moderate, grayish-white, smooth, glistening and spread rapidly over the surface of the medium. In four days, the growth was a dull white color, and the medium was slightly grayed.

Milk. No apparent change in cultures of plain milk occurred until the third day when there was evidence of slight clearing in the top layer of the medium. No coagulation occurred at any time and in 15 days the top one-half of the medium was cleared and had a slight greenish tinge. The consistency of the semi-transparent liquid was unchanged.

Azolitmin milk. Lavender colored azolitmin milk showed a pale blue layer on the surface in three days. In 15 days, one-half the medium had been cleared without coagulation. The pale blue zone still remained on the surface, but the remainder of the cleared liquid was colorless. The undigested portion showed no color change. No pink color occurred; therefore no acid was produced.

Methylene blue in milk. In this medium, digestion occurred as in the other milks, but no color change was noted until the eighth day when the color had disappeared in the cleared portion of the medium. The undigested portion remained the same color as the control.

Brom cresol purple in milk. Brom cresol purple in a concentration of 0.0016 percent, produces a light bluish color in milk. If the acidity is increased, the color turns yellow and if the medium becomes more alkaline a purple color develops. Cultures in this medium showed the usual digestion without coagulation and a reddish purple color occurred in the cleared liquid. This further substantiates the fact that no acid is produced from milk.

Nitrate reduction. A one percent potassium nitrate bouillon in fermentation tubes showed moderate growth in the open arm, but no growth in the closed arm and no gas was produced. A further test was made by growing the organism in a two percent peptone water containing 0 2 percent potassium nitrate. The cultures were tested at the end of seven days for nitrites, using the sulphanilic acid and naphthyl-amine-acetate test. Strong positive tests were secured for nitrites.

Carbon metabolism. To test for gas production from different carbon compounds, one percent solutions of dextrose, saccharose, maltose, lactose, mannitol, and glycerol were made up in a one percent Difco peptone water. Instead of using the ordinary U tubes, a simpler and more convenient type of fermentation tube was employed, consisting of a smaller test tube inverted within a larger one.

In all cases growth was abundant in the open arm but none occurred in the closed arm, or inner inverted tube. No gas was produced. Parallel cultures of *Bacterium coli* showed heavy clouding in the closed arm and gas produced from all six carbon compounds.

Acid production from carbon compounds. To determine accurately if acid was produced from carbon compounds the three sulphon phthalein indicators, brom cresol purple, brom thymol blue, and phenol red were used. These three indicators serve for a pH range from 5.2 to 8.4. Since the neutrality point is at pH 7.0 it is obvious that a series of media containing these indicators will serve to detect considerable acid or alkali production by the organism growing in culture.

A series of liquid media were prepared containing one percent Difco peptone and one percent of the following carbon compounds, dextrose, saccharose, lactose, maltose, mannitol, and glycerol. These media were prepared in triplicate. To one series brom cresol purple was added, in another brom thymol blue, and to the other, phenol red. The indicators were added at the rate of eight c.c. of a 0.2 percent alcoholic solution per liter of medium, giving a concentration of the indicator of 0.0016 percent. These media when prepared had a pH value of 6.8 to 7.0 as evidenced by the grass-green color of the medium containing brom thymol blue.

The series containing brom cresol purple were near the full alkaline color while the phenol red series were a very faint pink. In 24 hours after inoculation, there was slight acid production from dextrose indicated by a slight fading of the green color in the brom thymol blue series, and in four days, there was strong acid production, shown by a yellow color with all three indicators. With saccharose, acid production was not evident until two days after inoculation and in five days the brom thymol blue series were entirely yellow, but the brom cresol purple series still had a slight purple color. Not enough acid was produced from saccharose to give the full acid color to brom cresol purple, while with dextrose the purple color entirely disappeared. In the case of lactose and maltose considerable alkali was produced, indicated by the change to the alkaline color, while with mannitol and glycerol the color changed little from the controls. The foregoing tests demonstrated that acid is produced from dextrose and saccharose, but not from lactose, maltose, mannitol and glycerol.

A series of solid media were prepared using beef peptone agar and one percent of the following sugars; dextrose, saccharose, maltose and lactose. The three indicators enumerated above were used in the same concentration. Slant cultures were made. The color changes were the same as in the liquid media containing the same sugars. This further demonstrated that acid is produced from dextrose and saccharose, but not from maltose and lactose.

An additional series of carbon compounds were used for acid production in both liquid and solid media, using only the indicator, brom thymol blue. The following carbon compounds were used in a one percent concentration: arabinose, galactose, mannose, melezitose, raffinose, rhamnose, salicin, trehalose and xylose. The results were the same in both the liquid and solid media. Slight acid was produced from arabinose, galactose, mannose and xylose, indicated by the change from green to yellow color, while in the case of melezitose, rhamnose, raffinose, salicin and trehalose, the color became slightly more alkaline than the controls. A summary of acid production from the above named compounds is presented in table I.

Azolitmin sugar agars. A series of beef-peptone agars containing azolitmin and one percent of the following carbon compounds: dextrose, saccharose, maltose and lactose were prepared and the organism grown on them in slant cultures. In 24 hours the medium containing dextrose was pink along the slant and in 15 days, the color had entirely disappeared from the unslanted portion of the medium, but the slant was the same color as the control. The saccharose medium

TABLE I. ACID PRODUCTION FROM CARBON COMPOUNDS

Compound	Acid production
Arabinose	+
Dextrose	+
Galactose	+
Glycerol	—
Lactose	—
Maltose	—
Mannitol	—
Mannose	+
Melezitose	—
Raffinose	—
Rhamnose	—
Saccharose	+
Salicin	—
Trehalose	—
Xylose	+

showed slight pink along the slant in 2 days and in 15 days was similar to the dextrose series. There was no change to pink color in the maltose or lactose cultures, but the color turned dark blue thruout in 15 days .

Action on starch. Cultures of the organism growing on plates of beef-peptone agar to which starch had been added were flooded with a saturated solution of iodine in 50 percent alcohol. The entire medium turned a dark blue color, indicating that there had been no diastatic action on the starch.

Tests for indol and ammonia. Cultures were made in beef-peptone bouillon containing two percent Difco peptone to test for indol production. After seven days, there was no indol present when tested with potassium nitrite and sulphuric acid.

Cultures of the same medium were tested at the end of seven days for ammonia with Nessler's reagent and no positive test secured.

Cohn's solution. No growth occurred in Cohn's solution.

Uschinsky's solution. Slight growth occurred in 24 hours and in seven days, growth was good with a flocculent surface pellicle which settled on being disturbed. A greenish-yellow pigment was produced, first in the upper layer of the medium and later thruout the liquid.

Fermi's solution. Growth was slightly more vigorous in this medium at first than in Uschinsky's. The flocculent surface pellicle and the greenish-yellow pigment were produced as in Uschinsky's solution.

Toleration of sodium chloride. The organism produced slight clouding in tubes of neutral (pH 7) beef-peptone bouillon containing as high as six percent sodium chloride. The growth of the organism was inhibited by seven percent sodium chloride in the same medium.

Hydrogen-ion toleration. A series of beef-peptone bouillon were adjusted with normal hydrochloric acid to the following pH values, 6.0, 5.5, 5.0, 4.5 and 4.0. The pH values were determined colorometrically by comparison with color standards. Tube cultures were made in each medium. The results are presented in table II.

Table II shows that the organism tolerates a hydrogen-ion concentration of pH 5.0 and that the limit of tolerance is between pH 4.5 and pH 5.0.

TEMPERATURE RELATIONS

The organism grows in a wide range of temperatures. A series of slant and plate cultures were made on potato dextrose agar and incubated at the following temperatures: 0° C., 5°–6° C., 11°–12° C., room temperature (22°–23° C.), 25° C., 27.5°, 30° and 35° C. The organism

TABLE II. ACIDITY TOLERANCE AS INDICATED BY GROWTH IN MEDIA AT VARIOUS HYDROGEN-ION CONCENTRATION.

Incubation period	pH 6.0	pH 5.5	pH 5.0	pH 4.5	pH 4.0
1 day	good growth	good growth	light growth	no growth	no growth
2 days	heavy growth	heavy growth	moderate growth	no growth	no growth
5 days	heavy growth	heavy growth	moderate growth	no growth	no growth

grew slowly at 0° C., moderately at 5°–6° and 11°–12° C., while at room temperature, 25°, 27.5° and 30° C. good growth occurred. At 35° C. growth was very meagre. The maximum growth occurred at 25° to 30° C.

In determining the thermal death point, water suspensions of the organism in small thin-walled test tubes were exposed for 10 minutes to a series of temperatures in an electrically controlled water bath. After the exposure, the tubes were cooled at once and tests made by loop transfers to agar slants and poured dilution plates. A 10 minute exposure at 45° and 46° C. killed a large percentage of the organisms, 47° and 48° C. left few viable organisms and at 49°, 50° and 51° C. all were killed; therefore the thermal death point may be considered to be 49° C.

EFFECT OF FREEZING

Water suspensions of the organism were made and transferred aseptically to sterile, thin-walled, test tubes, which were placed in a mixture of ice and water in a compartment of a frigidaire refrigerator. Plates were poured when the suspensions were placed in the ice and water mixture. The suspensions did not freeze for 14 hours, but the mixture of ice and water was held at from 1° to 2° C. At the end of 14 hours, the tubes were frozen solid and remained frozen thruout the duration of the experiment except the two cases where noted. From time to time tubes were removed, thawed out and plates poured. The approximate number of bacteria per c.c. as indicated by plate counts were determined and the results are presented in table III.

In the second test evidently considerable growth had taken place at 1°–2° C. before freezing occurred. The fact that there were 700 viable bacteria per c.c. after 50 days in ice, indicates that some of the organisms were very resistant to freezing.

EFFECT OF SUNLIGHT

Plates of beef-peptone agar were poured from a suspension of the organism and exposed to the direct rays of the sun at 1 p. m. The

TABLE III. EFFECT OF FREEZING OF THE BACTERIA IN WATER.

Length of time frozen	Approximate number bacteria per ml.
0 (original suspension)	8,382,000
75 min. (after being 14 hrs. at 1°–2° C.)	69,375,000
10 hrs.	7,400,000
*9 days	3,908,000
11 days	624,000
*21 days	7,800
23 days	5,300
34 days	4,200
50 days	700

*Thawed for a few hours due to a faulty regulator in the Frigidaire refrigerator.

plates were placed on cracked ice and one-half of each plate covered with black paper. At intervals of 15 minutes plates were removed and incubated at room temperature. Plates exposed 15 minutes had a normal development of bacteria in them, while 30 minutes reduced the number of colonies developing in the unshaded portion of the plate one-half. In the plates exposed 45 and 60 minutes no bacteria developed in the unshaded portion while the shaded portion of the plates developed bacterial colonies normally.

RESISTANCE TO DESICCATION

Water suspensions of young vigorous growing agar slant cultures were made and a two millimeter loop of this suspension was smeared on a series of sterile cover glasses in a petri dish and allowed to dry. Tests were made from time to time for viable bacteria by dropping the cover glasses in a tube of beef-peptone bouillon and others on an agar slant so that the smear came in contact with the surface of the medium. Tests were made just as the smears were drying, after drying 15, 23 and 60 hours, respectively. These tests showed that the organism survived 60 hours' drying on sterile glass.

To determine the resistance to drying on seed of *Holcus sorghum*, seed were placed in petri dishes, moistened and sterilized in the autoclave. Water suspensions of the organism were poured on the sterilized seed and allowed to stand until the seed was thoroly wet. The excess suspension was then poured off and the dishes placed in a large sterilized glass culture chamber to dry. From time to time, seeds were removed and planted in agar poured plates. Tests thus far have shown the organism to be alive after three months drying on the seed. Furthermore it has been found that the organism lives over winter on or in seed of *Holcus sorghum*, showing that it is highly resistant to drying on or in the seed.

TAXONOMY OF THE CAUSAL ORGANISM

The organism causing holcus bacterial spot apparently differs from any previously described. It has been briefly characterized in a previous publication (16) and given the name *Bacterium holci* n. sp. (*Pseudomonas holci*). Bruyning (5) isolated two chromogenic bacteria from blighted sorghum which he named *Micrococcus aurantiacus sorghi*, producing a yellow pigment, and *Bacillus ruber ovatus*, producing a red pigment. He stated that the two organisms working symbiotically produced the disease known as sorghum blight. Since the symptoms of the disease under consideration are quite different and the organism concerned is in no way related to the organisms which he described, the two diseases should not be confused. Radais (21), working with apparently the same condition described by Bruyning found no specific organism associated with the disease, but attributed it to the action of yeasts.

The organism described in this bulletin as the cause of Holcus bacterial spot is obviously different from *Bacillus sorghi*, described by Burrill (1, 2) as the cause of a blight of sorghum. *Bacillus sorghi* as described by Burrill is a spore forming organism, does not liquefy gelatin and has an optimum temperature

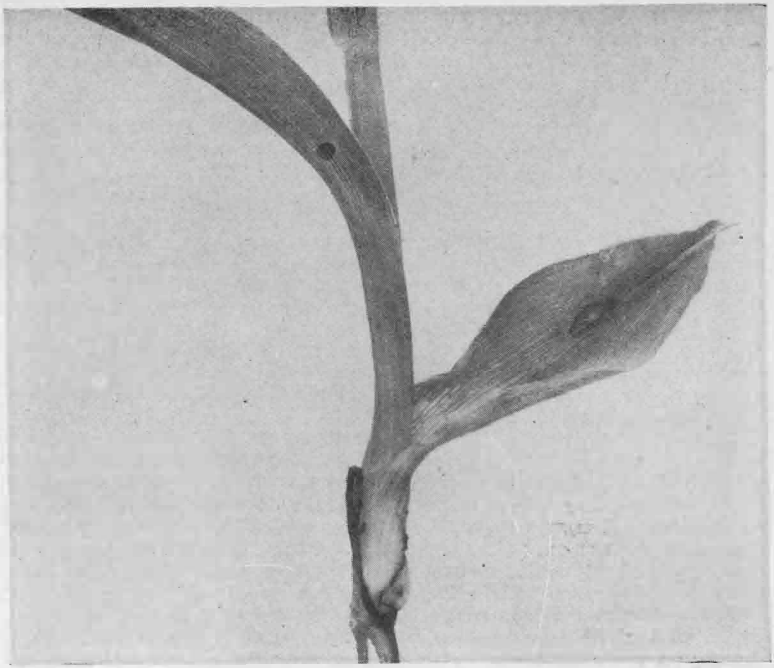

Fig. 3. Seedling *Holcus sorghum* plant grown in sterile soil showing primary lesion in the center of the first leaf. The lesion has a very small light center and a wide red border.

of 36° to 37° C., while the organism causing holcus bacterial spot is non-spore forming, liquefies gelatin and has an optimum temperature of 25° to 30° C. The fact that *Bacillus sorghi* produced spores differentiates it from the non-spore producing organism causing the disease described in this publication. The description of the organism which Burrill (3, 4) attributed to be the cause of a bacterial disease of corn is not adequate to allow a careful camparison with the organism causing holcus bacterial spot.

As previously stated, it is not now possible to identify accurately Burrill's diseases of sorghum and corn nor the organisms concerned. The evidence presented indicates that the condition which he described may have been partially caused by a combination of unfavorable environmental factors and saprophytic organisms. Since the symptoms of holcus bacterial spot differs materially from those of Burrill's diseases of sorghum and corn, and the organisms concerned are quite different, it seems logical to consider the disease described in this work to be different from the sorghum and corn bacterial disease described by Burrill.

The organism causing Holcus bacterial spot, in some respects, rather closely resembles *Bacterium andropogoni* described by Smith and Hedges (28) and Smith (29) as causing "Burrill's bacterial disease of broomcorn." However, a careful comparison shows several important differential characters. *Bacterium andropogoni* does not liquefy gelatin, reduce nitrates nor produce a green pigment; while the organism associated with holcus spot liquefies gelatin readily, reduces nitrates and produces a green water-soluble pigment.

The organism under consideration differs materially in morphological and cultural characters, as well as pathogenicity from *Phytomonas dissolvens* described by Rosen (25) as the cause of a stalk rot of corn. In the case of Stewart's disease of sweet corn (32), the causal organism is a yellow bacterium and a vascular parasite. Durrell (6) found no specific parasite associated with the purple leaf-sheath spot disease which he described on corn, but found that a number of more or less saprophytic organisms might produce the disease under favorable conditions.

The fact that the causal agent of holcus bacterial spot is a white bacterium at once differentiates it from the following yellow bacterial organisms causing diseases on gramineous hosts: *Bacterium translucens,* the cause of bacterial blight of barley (13, 14); *B. translucens* var. *undulosum,* the cause of black chaff of wheat (31); *B. translucens* var. *secalis,* the cause of bacterial blight of rye (22); *Aplanobacter agropyri,* the cause of a disease of western wheat grass (19, 20); and *Pseudomonas tritici,* the cause of a disease of wheat (12). The organism causing holcus bacterial spot differs materially in cultural and morphological characters from *Bacterium atrofaciens* described by McCulloch (17) as the cause of basal glume rot of wheat.

Manns (18) in 1909 isolated two bacterial organisms from a blade blight of oats which he claimed had a symbiotic relationship and named one *Bacillus avenae,* a white organism, and the other *Pseudomonas avenae,* a yellow organism. Elliott (8) in 1920, has shown that a white organism *Bacterium coronofaciens,* is the cause of "Halo Blight," of oats, which is in all probability the same disease with which Manns was working. Since *B. coronofaciens* has failed to infect *Holcus sorghum* and *Zea mays* when inoculated in the greenhouse

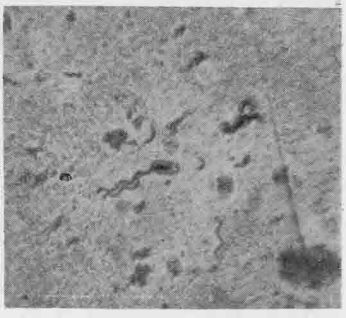

Fig. 4. Causal organism stained by Plimmer's method to show polar flagella. The light outer area surrounding the dark center is the result of the focus and not a capsule as it appears. Photo-micrograph x 1500.

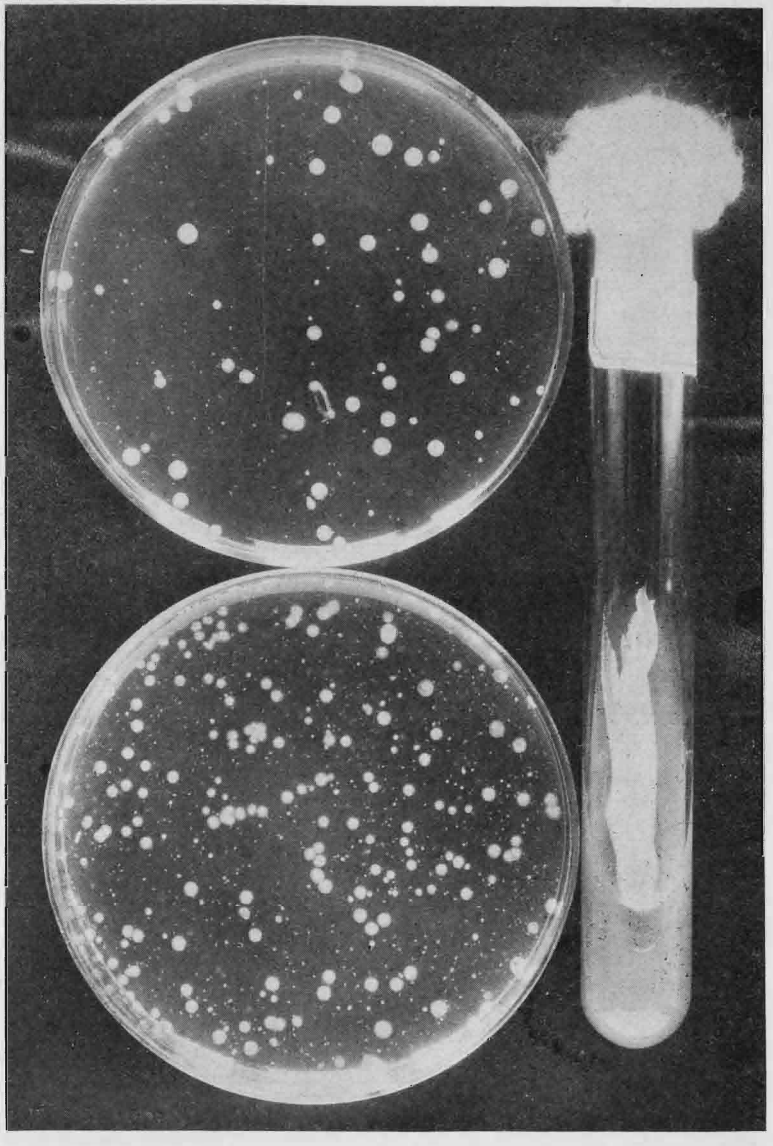

Fig. 5. Four day old plates of the causal organism on beef-peptone dextrose agar and two day old transfer to the same medium. These are plates from dilution two and three made by surface sterilizing a *Zea mays* lesion in mercuric chloride and crushing in sterile water.

and conversely the organism causing holcus bacterial spot does not attack *Avena sativa* it is quite apparent that they are two distinct organisms. The two organisms were also compared culturally and found to be different.

In 1924, Elliott (9) described a bacterial stripe disease of proso millet caused by a white bacterium, *Bacterium panici,* which somewhat resembles the organism that causes holcus bacterial spot. However she was not able to produce infection on *Holcus sorghum* and found the organism pathogenic only to proso millet. Culturally this organism differs from the organism causing holcus bacterial spot in that it grew in Cohn's solution, was non-fluorescent in Fermi's and Uschinsky's solutions, and did not produce acid with either dextrose or saccharose.

Rosen (26, 27) described a disease of foxtail (*Chaetochloa lutescens*) caused by a white bacterium which he named *Pseudomonas alboprecipitans.* He was able artificially to infect *Zea mays, Holcus sorghum, Triticum vulgare, Avenue sativa, Hordeum vulgare,* and *Secale cereale.* The organism causing holcus bacterial spot has not under repeated trials produced infection on *Triticum vulgare* or *Avena sativa,* and only sparingly on *Setaria glauca,* which according to Hitchcock (11) is synonymous with *Chaetochloa lutescens.* The organism causing holcus bacterial spot differs in the following cultural characters from *Pseudomonas alboprecipitans.* It produces no colorless zone around the colonies on agar plates and no diastatic action on starch, but liquefies gelatin and produces acid in the presence of some carbohydrates and a greenish pigment in Uschinsky's and Fermi's solutions.

This review of the cultural and morphological characters of the plant pathogens concerned in the diseases of gramineous hosts indicates clearly that the causal agent of Holcus bacterial spot is an undescribed organism.

TECHNICAL DESCRIPTION

PSEUDOMONAS HOLCI KENDRICK 1926, SYNONYM, *BACTERIUM HOLCI* KENDRICK

Cylindrical rods with rounded ends, single or in pairs; average measurement of individual rods .73μ by 2.13μ; motile by polar flagella (1-4); aerobic; non-spore forming; no capsules; involution forms not observed. Stains readily with Ziehl's carbol fuchsin and gentian violet; gram-negative.

Surface colonies on agar plates are round, smooth, glistening, raised or pulvinate, margin entire, internal structure finely amorphous; grayish white by reflected light, greenish fluorescent by transmitted light; produces greenish pigment in Fermi's and Uschinsky's solutions, less pronounced on beef-peptone, and veal infusion agars; no growth in Cohn's solution.

Gelatin liquefied; milk cleared in three weeks with no coagu-

lation nor acid production; nitrates reduced; no indol; no gas with dextrose, saccharose, lactose, maltose, mannitol nor glycerol; acid produced from arabinose, dextrose, galactose, mannose, saccharose and xylose; no diastatic action on starch. No growth in pH 4.5 beef-peptone broth, slight growth in pH 5.0 beef-peptone broth; growth inhibited by seven percent sodium chloride in pH 7.0 beef-peptone broth.

Grows 0°–35° C. optimum 25°–30° C.; thermal death point 49° C.; resistant to freezing in water; slight growth after 60 hours drying on cover glasses; resistant to desiccation on seed of *Holcus sorghum;* succumbs to 45 minutes exposure to direct sunlight.

Group number according to later chart adopted by the Society of American Bacteriologists[1] is 5322-3112-2232. Group number according to the old bacteriological chart is 211.2323133.

Pathogenic on *Holcus sorghum* L., *H. sorghum* var. *sudanensis* (Piper) Hitchc., *H. sorghum* var. *technicus* Bailey, *H. halepensis* L., *Zea mays* dent, sweet, flint and pop corn, *Pennisetum glaucum* (L.) R. Br., and *Chaetochloa lutescens* (Weigel) Stuntz.

RELATION OF PARASITE TO HOST TISSUE

Early in the study of this disease it became quite apparent that the organism never occurred in as great abundance in the lesions on the leaves of *Holcus sorghum, H. sorghum* var. *sudanensis* and *H. halepensis* as in lesions on leaves of *Zea mays* and *Pennisetum glaucum.* Often isolations from older lesions on the first named hosts yielded only a few typical bacterial colonies, or sterile plates. In every case where incipient lesions were cut in a drop of water on a slide, and placed under the low power of a microscope, bacteria oozed from the tissues in the center of the lesion. No trouble was encountered in securing cultures from such lesions. Similar lesions taken after the tissues had dried out and treated the same way often failed to reveal any bacteria oozing from the tissues and isolations resulted in relatively few bacterial colonies or sterile plates.

The foregoing phenomenon was repeatedly demonstrated from artificial inoculations by pouring plates from incipient lesions and from lesions on the same plants after the infected tissues had dried out. Many lesions from field material were examined under the microscope by cutting the lesions in a water drop on a slide and watching for the bacteria to ooze out. In practically every case where a large lesion with a rather large light area in the center was cut in a drop of water, bacteria oozed from the tissues, but in the case of smaller lesions with a very small light center or lesions entirely red, bacteria rarely oozed from the tis-

[1]Conn, H. J., et al. Report of the committee on the descriptive chart for 1919. Jour. Bact. 5:127-143, 1920.

sues. Cultures of the organism were easily secured from the lesions in which bacteria oozed from the tissues. Lesions of all sizes and ages on *Pennisetum glaucum* leaves showed bacteria oozing from the tissues in great abundance.

It seems probable that the bacteria are rather quickly arrested in their development in the case of the Holcus species, possibly by some toxic substance, and never become as abundant in the lesions as in the case of *Zea mays* and *Pennisetum glaucum*. Bacteria were not observed to ooze from the red discolored tissues bordering the lesions. This red color is apparently a substance produced when the tissues are injured by bacterial invasion and which diffuses thru the healthy tissue immediately surrounding the wound. No information has been obtained as to why the bacteria are apparently arrested in their development or die in the tissues in these hosts.

PATHOGENICITY STUDIES

Many inoculation experiments were made under greenhouse conditions upon various species of the family Gramineae, using three strains of the organism consistently. One strain was isolated from dent corn in the fall of 1924, one from a seedling plant of *Holcus sorghum*, and one a reisolation from a plant of *H. sorghum* var. *sudanensis* which had been previously inoculated with the strain from *Zea mays*. Reisolations have been made repeatedly and the identity of the causal organism determined. These three strains as well as others have been used in the study of the cultural characters of the organism.

In making inoculations the leaves of the plants were rubbed between wet fingers and then sprayed from an atomizer with a water suspension of the organism. The best results were obtained by using cultures 24 to 30 hours old. As soon as the plants were sprayed, they were placed in a large glass chamber covered with muslin, and provided with a layer of wet sphagnum moss in the bottom. The incubation period was found to be from two to three days. The plants were left in the inoculation chamber two days and then were removed to a greenhouse bench.

Inoculation tests on *Zea mays* have usually been made on young plants, and successful results have not always been obtained. When older plants were available for inoculation trials, more consistently successful results were obtained. Greenhouse studies and field observations indicate that *Z. mays* is more susceptible in its later stages of development, but doubtless environmental factors play an important role in the severity of the disease on this host. *Holcus sorghum*, *H. sorghum* var. *sudanensis* and *Pennisetum glaucum* are very susceptible in all stages, and markedly so in the seedling stage.

October 15, 1924, 30 young plants of dent corn were sprayed

thoroly with a suspension made from a culture of the organism isolated from *Zea mays*. The plants were incubated in the inoculation chambers mentioned previously. Three days after they were inoculated, 21 of the 30 plants showed abundant evidence of bacterial infection in the form of round, elliptical or irregular water-soaked areas on the leaves. The 28 plants sprayed with sterile water as controls remained healthy.

Again on October 22, 30 young plants of dent corn were sprayed with a suspension of the same strain and only three showed evidence of infection, while 19 young plants inoculated six days later showed no infection. November 5, three series of inoculations were made on young plants of *Zea mays, Holcus sorghum, H. sorghum* var. *sudanensis, Bromus inermis, Triticum aestivum* and *Avena sativa* using the organism mentioned above, one from *Holcus sorghum* and a reisolation from *Zea mays*. In two days, all plants of *Zea mays* as well as *Holcus sorghum* and *H. sorghum* var. *sudanensis* showed typical water-soaked lesions on the leaves. The lesions appeared on the last two named hosts as round to elliptical water-soaked areas, with a reddish green color which soon turned to red, or red bordered lesions. The three strains of the organism produced similar lesions and the organism was reisolated from all hosts. *Triticum aestivum, Avena sativa* and *Bromus inermis* showed no evidence of infection.

Another series of inoculations were made December 31, including *Holcus sorghum, H. sorghum* var. *sudanensis, H. halepensis, Arrhenatherum elatius, Dactylis glomerata, Lolium perenne, Phalaris sp. Agrostis palustris, Triticum aestivum, Phleum pratense, Festuca elatior* and *Bromus inermis*. Two days later, typical lesions were evident on the leaves of *Holcus sorghum, H. sorghum* var. *sudanensis*, and *H. halepensis*, but no indication of infection on the other grasses. The lesions on *H. halepensis* were the same as those on *H. sorghum* var. *sudanensis*. During the period from January 29 to February 9, the same series of plants were inoculated at four different times, fresh *Holcus sorghum, H. sorghum* var. *sudanensis* and *H. halepensis* plants being substituted in the series each time. Two strains of the organism were used. In each case, two days after inoculation, reddish-green, water-soaked, round to irregular lesions appeared abundantly on the leaves of the Holcus species, but no evidence of infection on the other grasses appeared.

Numerous inoculations were made on *Holcus sorghum* and *H. sorghum* var. *sudanensis* to test out the pathogenicity of reisolated strains. In practically every case, typical lesions resulted.

A series of plants including *Holcus sorghum, H. sorghum* var. *sudanensis, Pennisetum glaucum, Chaetochloa lutescens*, and the following varieties of *C. italica*; Japanese, Siberian, Hungarian

and Common were repeatedly inoculated with the different strains of the organism. In every case, typical infection occurred on the Holcus species and *Pennisetum glaucum,* but none on the varieties of *Chaetochloa italica.* The lesions appeared on the leaves of *Pennisetum glaucum* as round, elliptical, to linear irregular dark green water-soaked areas, which later became dark brown with a slight light greenish halo. There was no evidence of red color and the lesions were typical of those noted on plants growing in the field. This host was even more susceptible under greenhouse conditions than the Holcus species.

In order to test out the susceptibility of *Zea mays,* a series of inoculations were made on March 23, including the following varieties of (1) dent corn: Blue Flower, Minnesota Number 13, Io Jap Striped, Boone County White, Lancaster Sure Crop, Silver King, Golden Orange, Northwestern Dent, Wimple's Yellow Dent, St. Charles White, Pickett's Yellow Dent, Champion White Pearl, and Calico; (2) flint corn: Rainbow Flint and Rhode Island White Flint; (3) sweet corn: Howling Mob, Golden Bantam and Black Mexican; and (4) pop corn: Yellow Pearl and Jap Hulless. *Holcus sorghum* and *H. sorghum* var. *technicus* were included in this series to test the viability of the cultures used. All plants were in the seedling stage, and were divided into parallel series, using two strains of the organism. After three days incubation, infection was abundant on Holcus and slight on the following varieties of dent corn: Boone County White, Lancaster Sure Crop and Golden Orange. No evidence of infection occurred on the other varieties of *Zea mays.* The same plants were inoculated in parallel series again in 15 days, after the plants were 12 to 18 inches high. In addition, *Pennisetum glaucum* and the following varieties of *Chaetochloa italica*: Common, Hungarian, Siberian, Japanese and *Holcus sorghum* var. *technicus* (broomcorn) were included. After the usual incubation period, good infection showed on the *Holcus species* and *Pennisetum glaucum.* Slight infection occurred on the following varieties of dent corn: Wimple's Yellow Dent, Champion White Pearl, St. Charles White, Minnesota Number 13 and Pickett's Yellow Dent; flint corn: Rainbow Flint and Rhode Island White Flint; sweet corn: Golden Bantam.

Other pots of the varieties of *Zea mays* mentioned above were allowed to grow until they were in the tasseling stage. The plants never reached normal development because they were grown in pots, and were short and somewhat stunted. May 7, a series of these plants including the varieties named in the previous series were inoculated and after two days incubation, abundant evidence of infection showed on all varieties.

The following varieties of *Holcus sorghum* were tested in the greenhouse as to their relative susceptibility: Orange Cane,

White Kafir, Red Amber, Black Amber, Milo, White Durra, Standard Blackhull Kafir, Shallu, Shrock, Sunrise, Kaferita, Dwarf Hegeria, Dwarf Kafir, Dwarf Sumac, Red Kafir, Sumac, Kansas Orange, Pink Kafir and the two varieties of *H. sorghum* var. *technicus,* Evergreen and Acme. All proved susceptible. Under greenhouse conditions the Orange Cane, Kansas Orange, Shallu and the two varieties of broomcorn were perhaps slightly more susceptible than the others.

Fresh isolations were made from sweet corn and *Chaetochloa lutescens* June 25, 1925. These cultures proved pathogenic to *Holcus sorghum* and *Zea mays* and slight infection was produced on *Chaetochloa lutescens.* Numerous fresh isolations from *Holcus sorghum, H. sorghum* var. *sudanensis, H. halepensis, Pennisetum glaucum* and *Zea mays* made during July yielded a similar white bacterial organism which appeared to be identical with the organisms isolated previously. The pathogenicity of these isolations was proven by greenhouse inoculations.

July 16, the lower leaves of plants of sweet corn (Golden Bantam) and several selections of dent corn were sprayed with two strains of the organism. The weather was dry and very unfavorable for infection at this time. However, in four to five days, typical lesions were produced on a few of the lower older leaves in all cases.

A general summary of the pathogenicity studies shows that the bacterial leaf spot disease occurs on *Holcus sorghum, H. sorghum* var. *sudanensis, H. sorghum* var. *technicus, H. halepensis, Pennisetum glaucum, Zea mays* and *Chaetochloa lutescens.* The organisms from these different hosts have been repeatedly cross-inoculated from one host to the other. Consistent infection has been secured on the Holcus species and *Pennisetum glaucum,* but *Zea mays* has not responded as readily under greenhouse conditions as the first named hosts. Field observations have shown abundant infection on the lower leaves of *Zea mays* plants after June 25. Indications are that the age of the *Z. mays* plants and the environmental factors play an important part in the susceptibility of this host. *Chaetochloa lutescens* has shown only slight susceptibility in the field and greenhouse.

INFECTION

Since infection occurs when suspensions of the organism are sprayed on the leaves without wounding the tissues, it seems likely that infection takes place thru the stomata. Apparently the bacteria cause a necrosis of the tissues within a few hours after entering the plant. An examination of the lower and upper epidermis of the leaves showed an abundance of stomata on both surfaces. The bacteria are at first apparently intercellular, but soon cause a collapse of the tissues and become intracellular.

When young lesions were cut in drops of water on a slide and examined under the microscope, bacteria in abundance oozed from the intracellular spaces as a wavy, slow moving, cloud-like mass. Such sections showed that in the case of the Holcus species the bacteria were usually limited to a rather small central portion of the necrotic area. The red coloration associated with the lesions on Holcus species is a host reaction, apparently responsive to the injury produced by bacterial invasion. This color usually extends thru the tissues immediately surrounding the wound. The lesions appeared in cross-sections as saucer-shaped sunken areas.

Large *Holcus sorghum* plants growing in a greenhouse bench were sprayed early in May, 1925, with a suspension of bacteria isolated from a *Holcus sorghum* lesion. In a few days, typical leaf lesions occurred and 10 days after inoculation, small red spots appeared on the sheath enclosing the head of one of the plants. When the head emerged from the sheath, the glumes that were immediately under the infected area on the sheath appeared to be infected. They were dark red on the tip and the color soon involved the entire glume, while the normal glumes were still green. This color change should not be confused with the natural reddening of the glumes in the process of maturity of the seed, which starts at the base and extends towards the tip. Some of the diseased glumes were removed, surface-sterilized in mercuric chloride, and then washed in sterile water and successful reisolations secured. Transfers were made to agar slants and the pathogenicity of the reisolated bacterium proven on *Holcus sorghum*.

The presence of considerable moisture is apparently necessary for infection. Plants inoculated in a dry atmosphere resulted in only meagre infection. Natural infection in the field also occurs rarely during periods of dry weather.

SEED TRANSMISSION

While examining plants of *Holcus sorghum* in the field in the fall of 1924, many glumes were noted with red spots which resembled those on the leaves. This suggested the possibility that the causal organism might be carried over from year to year in the glumes which often remain with the seed. Several heads were cut from infected plants in this field and brought into the laboratory.

Seed from these heads were planted in pots of soil in the greenhouse. A red spot was noted on the first leaf of a few of the seedling plants (fig. 3). These spots resembled the smaller ones noted on the leaves in the field. Isolations were made from these lesions in the usual way and a white bacterial organism appeared in the plates which was apparently identical with the ones formerly isolated from the leaf lesions collected in the field and

which proved to be pathogenic when sprayed on species of Holcus in the greenhouse.

January 27, 1925, a few seeds from the same source were planted in a pot of soil which had been sterilized in an autoclave at 25 pounds pressure for 30 minutes on three consecutive days. Among the 17 seedlings grown in this soil, one showed a typical lesion on the first leaf identical with the lesions previously noted on seedlings in unsterilized soil.

A small package of *Holcus sorghum* seed of the White Milo variety was secured from Dr. L. W. Durrell, of the Colorado Agricultural Experiment Station early in January, 1925. Many of these seeds showed red lesions on the seed coat. An attempt was made to select diseased and healthy seed from this packet. The two selections were planted January 27, 1925, in pots of soil that had been sterilized in the autoclave as previously noted. A third planting was made from general seed from the same packet. From the 58 seeds that had shown red lesions, 54 seedling plants were produced and one plant showed a definite bacterial lesion on the first leaf. The 60 seeds that were apparently free from such lesions produced 56 plants and two had similar lesions on the first leaf. From the plantings of general seed, 156 seedlings resulted, and nine had lesions on the first leaf similar to those on the other plants. A total of 266 plants were grown from this seed and 12, or 4.5 percent, showed first leaf infection. This primary infection consists of small round or elliptical red spots or red bordered, light centered spots. These may be located in the middle or on the margin of the first leaf and are usually midway of the leaf.

May 13, 1925, a similar seed test was made with *Holcus sorghum* of the black and red amber varieties with seed secured from the Iowa State college seed laboratory. The age and source of the seed could not be determined. Of the 188 seedlings from the black amber variety, eight showed typical first leaf lesions, while four of the 219 red amber seedlings showed similar lesions.

Similar tests in pots of sterile soil were made on May 19, and June 1, 1925, using a large number of varieties. The seed was from different sources. The results are presented in table IV.

From the tests reported in table IV, which were made well beyond the time when seed of *Holcus sorghum* are planted, it is very evident that the organism may survive from one season to the next in or on the seed. A total of 11 out of 25 tests resulted in diseased seedlings, varying from 0.6 to 10.1 percent. The White Milo seed tested on May 19, were from the same packet that were tested on January 27, 1925. The early test showed 4.5 percent of infected seedlings while the latter showed 5.3 percent. In all cases where the seedlings were held for some time after the initial infection, secondary infection occurred on the upper leaves.

TABLE IV. *HOLCUS SORGHUM* SEED TEST OF MAY 19, 1925.

Variety	Source of seed	No. seedlings	No. infected	Percent infected
Black Amber	I.S.C. seed laboratory	169	0	0
Red Amber	" " "	159	1	0.6
Orange Cane	" " "	193	0	0
White Durra	" " "	124	0	0
Dakota Red	Kansas	197	0	0
Black Amber	"	152	0	0
Red Amber	"	143	1	0.7
Kansas Orange	"	159	0	0
White Milo	Colorado	189	10	5.3
	June 1, 1925.			
Dwarf Hegari	Kansas	84	2	2.38
Kansas Orange	"	100	2	2.0
Shrock	"	134	0	0
White Africa	"	125	0	0
Dorso	"	133	0	0
Sumac	"	145	0	0
Dwarf Kafir	"	93	0	0
Reed Kafir	"	89	9	10.1
Dwarf Sumac	"	133	0	0
Sunrise	"	117	0	0
Blackhull Kafir X Sourless	"	91	0	0
Kaferita	"	174	5	2.8
Pink Kafir	"	169	5	2.9
Red Kafir	"	134	7	5.2
Kansas Orange selection	"	248	4	1.6
Kafir X Feterita	"	152	1	0.65

OVERWINTERING

From the foregoing discussion, apparently the causal organism may overwinter in or on *Holcus sorghum* seed. In testing the effect of freezing on the causal organism, it was found that it survived 50 days freezing in water. In the course of the 50 days, the suspensions were thawed only twice for a very short time. The organisms that survived the 50 days freezing were equally as vigorous on culture media as those that were carried under normal laboratory conditions. Laboratory tests have also shown that the bacteria grew at 0° C. These two facts indicate that the organism might easily survive the winter in the field in old infected plants or on decaying organic matter. It is entirely possible that during a period of warmer weather, the organism continues growth on the dead and decaying host plants in the soil.

Field observations during the spring of 1925 at Ames, Iowa, indicate that the organism does live over in the soil. Late in the fall of 1924, an examination was made of the Forage Crop Experimental plot at Ames. Holcus bacterial spot was found abundantly on *Holcus sorghum, H. sorghum* var. *sudanensis* and *Pennisetum glaucum*. These same plots were again used for forage crops in 1925 and observations were made on the plants in the seedling stage before much if any secondary infection could have

taken place. The majority of the Holcus varieties were planted in hills, 18 to 24 inches apart in two-foot rows. There were three to eight plants per hill. June 13, 1925, the plots were first examined for bacterial infection. The plants were 6 to 10 inches high and still had the first leaves attached to the plants. Typical round, elliptical to irregular red lesions were noted on the first leaves of a considerable number of plants. In many cases where infection occurred in a hill, a spot could be found on the first leaf of one or more plants. The infected hills in each variety were counted and the data are presented in table V.

Besides the varieties reported in table V several other varieties were planted in the drill in adjacent rows and the plants were not counted. *Holcus sorghum,* varieties Ribbon Cane and Orange Cane, *H. sorghum* var. *sudanensis* and *Pennisetum glaucum* were all in rows in such large numbers that counts were not attempted, but examination showed approximately as heavy seedling infection as in case of the above counted varieties. Some of this infection very likely came from infected seed, but since in the numerous seed tests in sterile soil, never more than 10 and usually only 1 to 2 percent were found infected, it is quite evident that a relatively large percentage of the seedling infection in these plots came from the soil.

No evidence that the organism is harbored on seed of *Zea mays* was obtained, since seedling infection was not noted. June 25, 1925, bacterial lesions were observed on the lower leaves of *Z. mays* var. *saccharata* in an experimental field at Ames, Iowa. The plants were about ready to tassel. On further examination of fields of *Z. mays* var. *indentata* near Ames the following week, several were found in which the plants showed similar lesions in abundance on the lower leaves. Since there is no evidence that the seed of *Z. mays* carries the causal organism, and considering the late appearance of the first infection, the primary lesions may have come from the soil or from some other host plant.

CONTROL

Since the causal agent of Holcus bacterial spot is apparently carried with Holcus seed and also overwinters in the soil, seed selection and crop rotation are suggested as possible control

TABLE V. SEEDLING INFECTION IN *HOLCUS SORGHUM* PLOTS. JUNE 13, 1925.

Variety	Total number of hills	Number showing infection	Percentage of infection
Jap Sugar Cane	55	37	67.3
Evergreen Broomcorn	46	12	26.1
Feterita	36	9	25.0
White Kafir	43	14	30.2
Higeria	53	15	28.3

measures. In the case of Holcus species, seed should be selected from fields that show little if any bacterial infection. The fact that the causal organism also lives over in the soil will necessitate a system of rotation whereby Holcus species should not be planted on land that had Holcus on it the previous year.

There is no evidence that the disease is carried over on seed of *Zea mays*. In so far as is known, infection on *Zea mays* comes from the soil and other susceptible hosts growing in or near fields of *Zea mays*. Clean cultivation, especially the destruction of volunteer Holcus species in fields of *Zea mays* is suggested. Crop rotation should be practiced with *Zea mays* wherever possible. Land which had a bad infestation of Holcus bacterial spot should not be planted to corn or any of the susceptible hosts for at least one year and longer if possible.

SUMMARY

The Holcus bacterial spot herein described is a leaf spot disease of *Holcus sorghum* (sorghum), *H. sorghum* var. *sudanensis* (sudan grass), *H. sorghum* var. *technicus* (broomcorn), *H. halepensis* (Johnson grass), *Zea mays* (corn), *Pennisetum glaucum* (pearl millet) and *Chaetochloa lutescens* (foxtail).

Since the symptoms of this spot disease as well as the causal agent differ materially from Burrill's "Sorghum blight" and its causal agent, the name "Holcus bacterial spot" is suggested for the spot disease herein described.

Field observations and greenhouse inoculations revealed the following as hosts for the disease: 22 varieties of *Holcus sorghum* and *H. sorghum* var. *technicus, H. sorghum* var. *sudanensis, H. halepensis, Pennisetum glaucum;* 20 varieties of *Zea mays,* 11 of which are dent corn, 2 flint corn, 6 sweet corn and 1 pop corn, and *Chaetochloa lutescens.*

The following list of plants have not become infected under repeated greenhouse inoculations: *Triticum vulgare, Avena sativa, Bromus inermus, Arrhenatherum elatius, Dactylis glomerata, Lolium perenne, Phalaris,* sp., *Agrostis, Phleum pratense, Festuca elatior,* and the following varieties of *Chaetochloa lutescens*: Japanese, Siberian, Common, Hungarian and Broomcorn.

The disease on the Holcus species is characterized by light centered, red bordered, round, elliptical, lesions on the leaves. Very small lesions are red thruout. Often the spots are so numerous as to cause the death of the entire leaf. On *H. sorghum* variety Shallu, the lesions have a dark brown instead of a red border, while on *Pennisetum glaucum* and *Chaetochloa lutescens* the spots are a dark brown thruout with a narrow light green halo.

The lesions on the leaves of *Zea mays* are similar in shape to those on the Holcus species, but have a light to darker brown center and a narrow reddish brown border. Marginal infection often results in long necrotic areas along the edge of the leaf.

The disease has been known to occur in Iowa every year since 1916. The spots are often so numerous on the leaves of Holcus species and *Zea mays* as seriously to impair the vitality of the plant.

The causal organism is a white fluorescent, cylindrical, polar flagellate bacterium, previously described and named *Pseudomonas holci* (Synonym, *Bacterium holci*, n. sp.), The colonies on agar are round, grayish white in reflected light and slightly greenish fluorescent in transmitted light. Gelatin is liquefied and nitrate reduced.

Acid is produced from dextrose, saccharose, arabinose, galactose and xylose. Greenish pigment formation occurs in Fermi's and Uschinsky's solutions.

The organism grows from 0°to 35° C., optimum 25° to 30° C., survived 50 days freezing in water, 6 hours drying on glass, and grew vigorously after three months drying on seed of *Holcus sorghum.*

The group number according to the later chart adopted by the Society of American Bacteriologists is 5322-31121-2232, while according to the old chart the number would be 211.2323133.

Infection is apparently stomatal. The organism is at first intercellular, but soon causes a collapse of the tissues and becomes intracellular. The red color produced in the case of the Holcus species is a host reaction, resulting from the injury produced by the invading bacteria.

The bacteria are apparently rather quickly inhibited in their development in the tissues of Holcus which may possibly be due to some toxic substance produced in the injured tissue. Older lesions on Holcus species have failed to yield the organism while lesions of all ages on *Zea mays* and *Pennisetum glaucum* yield an abundance of the causal bacteria.

Of 25 tests of *Holcus sorghum* seed made in sterile soil, including 23 varieties and selections, seedling infection of from 0.6 to 10.1 percent occurred in 11 of these tests. The causal organism may be carried over winter in or on *Holcus sorghum* seed. Evidence is presented which indicates that the organism also overwinters in the soil.

No evidence of the causal agent being carried on seed of *Zea mays* is presented. Initial infection on this host comes from the soil or other hosts growing in close proximity.

As a control measure, selection of Holcus seed from fields free from the disease is recommended, destruction of volunteer Holcus plants growing in and near *Zea mays* fields and crop rotation are suggested.

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